UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: August 20, 2008

SUBJECT: Secondary Review of Contractor's (DynCorp Systems & Solutions LLC, a CSC Company) Efficacy Review for Carbosan 7.5,
EPA File Symbol 6836-GGE;
DP Barcode: D353295

FROM: Lorilyn M. Montford
Product Science Branch
Antimicrobials Division (7510P)

THRU: Dr. Tajah Blackburn, Team Leader
Product Science Branch
Antimicrobials Division (7510P)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

APPLICANT: Lonza Inc.
90 Boroline Rd,
Allendale, NJ 07401

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Didecyl dimethyl ammonium carbonate and Didecyl dimethyl ammonium bicarbonate	7.5%
Inert Ingredients	92.5%
Total	100.0%

I BACKGROUND

The product, Carbosan 7.5 (EPA File Symbol 6836-GGE), is a new product. The applicant requested to register the product as a sanitizing rinse and deodorizer for use on hard, non-porous surfaces in household, food processing, commercial, industrial, and institutional environments. The label claims that the product is effective in the presence of 500 ppm hard water. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant's representative (dated May 8, 2008), four studies (MRID 474263-04 through -07), Statements of No Data Confidentiality Claims for all four studies, and the proposed label.

II USE DIRECTIONS

The product is designed for sanitizing pre-cleaned, hard, non-porous, non-food contact surfaces such as ultrasound transducers, probes, mammography compressor plates, and salon/ barber tools and instruments. The product label did not include directions for use of the product to sanitize pre-cleaned, hard, non-porous, non-food contact surfaces.

The product is for use in sanitizing pre-cleaned, hard, non-porous, food contact surfaces such as appliance exteriors, beer fermentation and holding tanks, blenders, bottling or premix dispensing equipment, citrus processing equipment and holding tanks, coffee pots, coffee urns, cooking utensils, coolers, counters, chopping blocks, cutlery, cutting boards, dishes, drinking fountains, eating utensils, exhaust fans, frozen beverage machines, glassware, highchairs, ice chests, ice machines, kitchen equipment, food storage containers, refrigerated storage and display equipment, silverware, sinks, stoves, tables, tableware, tea dispensers, utensils, and water dispensers. The product label indicates that the product may be used on hard, non-porous surfaces including chrome, enamel, fiberglass sinks, glass, glazed ceramic, glazed enamel, glazed porcelain, laminated surfaces, metal, plastic (e.g., polystyrene, polypropylene), sealed granite, sealed limestone, sealed marble, sealed slate, sealed stone, sealed terra cotta, sealed terrazzo, stainless steel, vinyl and plastic upholstery, and finished woodwork. Directions on the proposed label provided the following information regarding preparation and use of the product as a sanitizing rinse: Prepare a use solution by adding 1 ounce of product to 2½-4 gallons of water (150-240 ppm active quat; a 1:275-1:512 dilution). Prior to application, remove gross food particles and soil by a pre-flush, pre-scrape, or pre-soak. Then thoroughly wash or flush surfaces with a good detergent or compatible cleaner, followed by a potable water rinse. Apply use solution to pre-cleaned surfaces with a cloth, mop, sponge, sprayer, or by immersion, thoroughly wetting surfaces. Surfaces must remain wet for at least 1 minute. Allow to drain and air dry. Do not rinse.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Sanitizers (For Non-Food Contact Surfaces)

The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface. The test surface(s) should represent the type(s) of surfaces recommended for

treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces)

Sanitizing rinses may be formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, or anionic detergent-acid formulations. The effectiveness of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method. Data from the test on 1 sample from each of 3 different product lots, one of which is at least 60 days old against *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) are required. When the effectiveness of the product in hard water is made, all required data must be developed at the hard water tolerance claimed. Acceptable results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and the percentage reduction over the control. Furthermore, counts on the number controls for the product should fall between 75 and 125 x 10^6/mL for percent reductions to be considered valid. Label directions for use must state that a contact time of at least 1 minute is required for sanitization. A potable water rinse is not required (to remove the use solution for the treated surface) for products cleared for use on food contact surfaces under the Federal Food, Drug, and Cosmetic Act. Label directions must recommend a potable water rinse (to remove the use solution from the treated surface) under any other circumstances.

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces; Additional Bacteria)

There are cases where an applicant requests to make claims of effectiveness against additional bacteria for a product that is already registered as a sanitizing rinse for previously cleaned, food contact surfaces. Confirmatory test standards would apply. For sanitizing rinses for previously cleaned, food contact surfaces, 2 product samples, representing 2 different product lots, must be tested against each additional microorganism. Results must show a bacterial reduction of at least 99.999% in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and the percentage reduction over the control.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 474263-04 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352) for Carbosan 7.5%, by Becky Lien. Study conducted at ATS Labs. Study completion date – June 22, 2007. Project Number A04937.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Three lots (Lot Nos. NB5794-86A, NB5794-86B, and NB5794-86C) of the product, Carbosan 7.5%, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended

for Inanimate Non-Food Contact Surfaces (ASTM E1153). At least one of the product lots tested (i.e., Lot No. NB5794-86C) was at least 60 days old at the time of testing. Testing was conducted on May 2, 2007 and June 12, 2007. Use solutions were prepared by adding 1.0 mL of the product and 374.0 mL of 500 ppm AOAC synthetic hard water (titrated at 495-503 ppm; a 1:375 dilution). Five sterile glass square carriers per product lot per microorganism were inoculated with 20 µL of a 48±4 hour old suspension of *Staphylococcus aureus*, or 30 µL of a 48±4 hour old suspension of *Klebsiella pneumoniae*. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried for 20 minutes at 36.0°C at 40-42% relative humidity. Each carrier was transferred to a sterile vessel and was exposed to 5.0 mL of the use solution for 1 minute at 20-26.0°C. After exposure, 20 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was added to each vessel and the vessels were rotated vigorously on an even plane to suspend the surviving microorganisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All plates were incubated for approximately 46.5 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, carrier quantitation, dry carrier count, inoculum count, and neutralization confirmation.

2. MRID 474263-05 "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces," Test Organism: *Listeria monocytogenes* (ATCC 19117) for Carbosan 7.5%, by Becky Lien. Study conducted at ATS Labs. Study completion date – May 14, 2007. Project Number A04938.

This study was conducted against *Listeria monocytogenes* (ATCC 19117). Two lots (Lot Nos. NB5794-86A and NB5794-86B) of the product, Carbosan 7.5%, were tested. The laboratory report referenced the Sanitizer Test from DIS/TSS-10 and the Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (ASTM E1153). Use solutions were prepared by adding 1.0 mL of the product and 374.0 mL of 500 ppm AOAC synthetic hard water (titrated at 495 ppm; a 1:375 dilution). Five sterile glass square carriers per product lot were inoculated with 20.0 µL of a 48±4 hour old suspension of *Listeria monocytogenes*. The inoculum was spread to within 1/8 inch of the edges of the carrier. The carriers were dried for 25 minutes at 36.0°C at 40% relative humidity. Each carrier was transferred to a sterile vessel and was exposed to 5.0 mL of the use solution for 1 minute at 20°C. After exposure, 20.0 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 was added to each vessel and the vessels were rotated vigorously on an even plane to suspend the surviving microorganisms. Within 30 minutes of the addition of the neutralizer, 1.00 mL aliquots of the 10^0 and 10^{-1} dilutions were plated in duplicate on tryptic soy agar with 5% sheep's blood. All plates were incubated for 52 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, carrier quantitation, dry carrier count, inoculum count, and neutralization confirmation.

3. MRID 474263-06 "Germicidal and Detergent Sanitizing Action of Disinfectants," Test Organisms: *Escherichia coli* O157:H7 (ATCC 35150), *Klebsiella pneumoniae* (ATCC 4352), and *Salmonella typhimurium* (ATCC 23564) for Carbosan 7.5%, by Anne Stemper. Study conducted at ATS Labs. Study completion date – May 21, 2007. Project Number A04913.

This study was conducted against *Escherichia coli* O157:H7 (ATCC 35150), *Klebsiella pneumoniae* (ATCC 4352), and *Salmonella typhimurium* (ATCC 23564). Two lots (Lot Nos.

NB5794-86A and NB5794-86B) of the product, Carbosan 7.5%, were tested using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Testing was conducted on April 24, 2007 and May 4, 2007. Use solutions were prepared by adding 1.5 mL of the product and 748.5 mL of 500 ppm AOAC synthetic hard water (or the equivalent) (titrated at 499-507 ppm; a 1:500 dilution). A 99-mL aliquot of the use solution for each test organism was transferred to a sterile, 250-300 mL Erlenmeyer flask and placed in a water bath at 25.0°C. One-mL bacterial suspension was added to each flask. One-mL aliquots of the bacterium-product mixture were transferred to 9 mL of Letheen Broth containing 0.07% Lecithin and 0.5% Tween 80 exactly 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots were plated in tryptone glucose extract agar. All plates were incubated for 48±4 hours at 35-37°C. Plates for testing performed on May 4, 2007 were stored for 1 day at 2-8°C prior to reading. Following incubation or incubation and storage, the colonies were counted. Controls included those for purity, sterility, viability, numbers count, and neutralization confirmation.

Note: The applicant provided the data for a failed study set up on April 24, 2007. In that trial, testing for Lot No. NB5794-86B against *Salmonella typhimurium* failed to demonstrate a 99.999% bacterial reduction and the numbers control for *Salmonella typhimurium* exceeded the acceptance criterion of 75-125 x 10^6 CFU/mL stated in the protocol. Thus, this study was invalid. See Attachment I of the laboratory report. Testing was repeated on May 4, 2007 for Lot No. NB5794-86B against *Salmonella typhimurium*.

4. MRID 474263-07 "Germicidal and Detergent Sanitizing Action of Disinfectants," Test Organisms: *Vibrio cholerae* (ATCC 11623), *Shigella sonnei* (ATCC 25931), and *Yersinia enterocolitica* (ATCC 23715) for Carbosan 7.5%, by Anne Stemper. Study conducted at ATS Labs. Study completion date – July 31, 2007. Project Number A04895.

This study was conducted against *Vibrio cholerae* (ATCC 11623), *Shigella sonnei* (ATCC 25931), and *Yersinia enterocolitica* (ATCC 23715). Two lots (Lot Nos. NB5794-86A and NB5794-86B) of the product, Carbosan 7.5%, were tested using the AOAC Germicidal and Detergent Sanitizing Action of Disinfectants Method (modified) as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. Testing was conducted on April 26, 2007, May 25, 2007, and June 15, 2007. Use solutions were prepared by adding 1.5 mL of the product and 748.5 mL of 500 ppm AOAC synthetic hard water (titrated at 499 ppm; a 1:500 dilution). A 99-mL aliquot of the use solution for each test organism was transferred to a sterile, 250 mL Erlenmeyer flask and placed in a water bath at 25.0°C. One-mL bacterial suspension was added to each flask. One-mL aliquots of the bacterium-product mixture were transferred to 9 mL of neutralizer blanks exactly 30 seconds after the addition of the bacterial suspension. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots were plated in tryptic soy agar with 5% sheep's blood. All plates were incubated for 48±4 hours at 35-37°C. Plates were stored for 2 days at 2-8°C prior to reading. Following incubation and storage, the colonies were counted. Controls included those for purity, sterility, viability, numbers count, and neutralization confirmation.

Note: The applicant provided the data for a failed study set up on April 26, 2007. In that trial, both product lots tested against *Vibrio cholerae* failed to demonstrate a 99.999% bacterial reduction and the numbers control for *Vibrio cholerae* was below the acceptance criterion of 75-125 x 10^6 CFU/mL stated in the protocol. Thus, this study was invalid. See Attachment I of the laboratory report. Testing was not repeated. Per request by the applicant, testing against *Vibrio cholerae* was cancelled prior to the generation of valid data.

Note: The applicant provided the data for a failed study set up on April 26, 2007. In that trial, both product lots tested against *Shigella sonnei* failed to demonstrate a 99.999% bacterial reduction and the numbers control for *Shigella sonnei* was below the acceptance criterion of 75-125 x 10^6 CFU/mL stated in the protocol. Thus, this study was invalid. See Attachment I of the laboratory report. Testing was repeated on May 25, 2007, using a 1:375 dilution of the product (instead of a 1:500 dilution of the product). In that trial, both product lots tested against *Shigella sonnei* failed to demonstrate a 99.999% bacterial reduction and the numbers control for *Shigella sonnei* exceeded the acceptance criterion of 75-125 x 10^6 CFU/mL stated in the protocol. Thus, this second study was invalid. See Attachment II of the laboratory report. Testing was repeated on June 15, 2007, using a 1:375 dilution of the product. In that trial, the numbers control for *Shigella sonnei* was below the acceptance criterion of 75-125 x 10^6 CFU/mL stated in the protocol. Thus, this study was invalid. See Attachment III of the laboratory report. Per Sponsor request, all *Shigella sonnei* testing is considered invalid. Per request by the applicant, testing against *Shigella sonnei* was cancelled prior to the generation of valid data.

V RESULTS

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/carrier)		
474263-04	*Staphylococcus aureus*	NB5794-86A	<4.17 x 10^1	4.79 x 10^6	>99.9
		NB5794-86B	<2.51 x 10^1	4.79 x 10^6	>99.9
		NB5794-86C	<2.5 x 10^1	3.72 x 10^6	>99.9
	Klebsiella pneumoniae	NB5794-86A	>1.12 x 10^4	9.33 x 10^5	<98.8
		NB5794-86B	>2.40 x 10^3	9.33 x 10^5	<99.7
474263-05	*Listeria monocytogenes*	NB5794-86A	<2.51 x 10^1	1.07 x 10^5	>99.9
		NB5794-86B	<2.51 x 10^1	1.07 x 10^5	>99.9
474263-06	*Escherichia coli* O157:H7	NB5794-86A	3.1 x 10^2	1.15 x 10^8	99.999
		NB5794-86B	9 x 10^1	1.15 x 10^8	99.999
	Klebsiella pneumoniae	NB5794-86A	<1 x 10^2	1.19 x 10^8	>99.999
		NB5794-86B	<1 x 10^2	1.19 x 10^8	>99.999
	Salmonella typhimurium Test Date: 4/24/07	NB5794-86A	1.1 x 10^2	1.45 x 10^8	99.999
	Salmonella typhimurium Test Date: 5/04/07	NB5794-86B	<6 x 10^1	7.8 x 10^7	>99.999
474263-07	*Yersinia enterocolitica*	NB5794-86A	<1 x 10^2	8 x 10^7	>99.999
		NB5794-86B	<1 x 10^2	8 x 10^7	>99.999

VI CONCLUSIONS

1. The submitted efficacy data (MRID 474263-04 and -05) support the use of the product, Carbosan 7.5, as a sanitizer against *Staphylococcus aureus* and *Listeria monocytogenes* on pre-cleaned, hard, non-porous, non-food contact surfaces in the presence of 500 ppm hard water for a contact time of 1 minute at a 1:375 dilution. A bacterial reduction of at least 99.9 percent over the parallel control was observed within 5 minutes. At least one of the product lots tested against *Staphylococcus aureus* was at least 60 days old at the time of testing. The carrier quantitation counts for the tested microorganisms met the laboratory acceptance criterion of 2.0 x 10^4 CFU/carrier. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2. The submitted efficacy data (MRID 474263-04) do not support the use of the product, Carbosan 7.5, as a sanitizer against *Klebsiella pneumonia* on pre-cleaned, hard, non-porous, non-food contact surfaces in the presence of 500 ppm hard water for a contact time of 1 minute at a 1:375 dilution. A bacterial reduction of at least 99.9 percent over the parallel control was not observed within 1 minute. At least one of the product lots tested was at least 60 days old at the time of testing. The carrier quantitation counts for the tested microorganism met the laboratory acceptance criterion of 2.0 x 10^4 CFU/carrier. Neutralization confirmation testing met the acceptance criterion of growth within 1 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

3. The submitted efficacy data support the use of the product, Carbosan 7.5, as a sanitizing rinse against the following microorganisms on pre-cleaned, hard, non-porous, food contact surfaces in the presence of 500 ppm hard water for a contact time of 30 seconds at a 1:500 dilution:

Escherichia coli O157:H7	MRID 474263-06
Klebsiella pneumoniae	MRID 474263-06
Salmonella typhimurium	MRID 474263-06
Yersinia enterocolitica	MRID 474263-07

A bacterial reduction of at least 99.999 percent over the parallel control was observed within 30 seconds. The numbers controls for the tested microorganisms met the laboratory acceptance criterion of 75-125 x 10^6 CFU/mL, with one exception. The numbers control for testing Lot No. NB5794-86A against *Salmonella typhimurium* exceeded the acceptance criterion of 75-125 x 10^6 CFU/mL stated in the protocol. [Note: The exceedance of the numbers control is acceptable for the data presented for Lot No. NB5794-86A against *Salmonella typhimurium*. Substitution of 125 x 10^6 in the percent reduction calculation gave results of at least 99.999% bacterial reduction.] Neutralization confirmation testing met the acceptance criterion of growth within ±1.0 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

VII RECOMMENDATIONS

1. The proposed label claims that the product, Carbosan 7.5, is an effective sanitizing rinse on pre-cleaned, hard, non-porous, food contact surfaces against the following microorganisms in the presence of 500 ppm hard water for a 1-minute contact time at a 1:512 dilution:

Escherichia coli O157:H7 (ATCC 33150)
Klebsiella pneumoniae (ATCC 4352)
Salmonella typhi[murium] (ATCC 23564)
Yersinia enterocolitica (ATCC 23715)

Because efficacy test data were developed using a 1:500 dilution of the product, these claims are acceptable only if the dilution rates identified on page 9 of the proposed label are corrected to read "1 ounce per 3.9 gallons of water" instead of "1 ounce per 4.0 gallons of water." In addition, the ATCC number for *Escherichia coli* O157:H7 must be changed to read (ATCC 35150).

2. The proposed label claims that the product, Carbosan 7.5, is an effective sanitizing rinse on pre-cleaned, hard, non-porous, food contact surfaces against *Shigella sonnei* (ATCC 25931) in the presence of 500 ppm hard water for a 1-minute contact time at a 1:512 dilution. This claim is not supported by the submitted data. References to *Shigella sonnei* must be removed from the proposed label.

3. The proposed label claims that the product, Carbosan 7.5, is an effective sanitizing rinse on pre-cleaned, hard, non-porous, food contact surfaces against the following microorganisms in the presence of 500 ppm hard water for a 1-minute contact time at a 1:512 dilution:

Campylobacter jejuni (ATCC 33560)
Escherichia coli (ATCC 11229)
Listeria monocytogenes (ATCC 19115)
Staphylococcus aureus (ATCC 6538)
Vibrio enterocolitica (ATCC 11623)

No data were provided to support these claims. References to the microorganisms listed above must be removed from the proposed label.

4. The proposed label states that the product may be used to sanitize pre-cleaned, hard, non-porous, non-food contact surfaces such as ultrasound transducers, probes, mammography compressor plates, and salon/ barber tools and instruments. As noted in the "Conclusions" section of this efficacy report, DIS/TSS-10 standards were not fully met as the product failed to be effective against *Klebsiella pneumonia*. References to the above surfaces must be removed from the proposed label.

5. Page 14 of the proposed label provides directions for three sanitizing applications, specifically Entryway Sanitizing Systems, Shoe Bath Sanitizer Directions, and Shoe Foam Sanitizer Directions. The dilution rates for these applications are:

0.125 ounce per gallon	equivalent to 200 ppm active
0.25 ounce per gallon	equivalent to 400 ppm active
0.50 ounce per gallon	equivalent to 800 ppm active
0.75 ounce per gallon	equivalent to 1,200 ppm active

Please confirm these dilution rates. Dilution rate information on page 9 of the proposed label indicates that a 0.25 ounce per 1 gallon of water use solution is equivalent to a 150 ppm active solution (not a 400 ppm active solution).

6. Information on the packet label on page 19 of the proposed label is incorrect, including the dilution rate, the page reference to the dilution chart, and the active ingredient concentration. Please review and correct this information.

7. The following changes are required on the proposed label:

- On page 5 of the proposed label, change "*Salmonella typhi*" to "*Salmonella typhimurium*" to correspond with the efficacy data and the ATCC number.

- On pages 10 and 11 of the proposed label, change "clean water" to read "potable water."

- On page 16 of the proposed label, add instructions to the "Storage and Disposal" section that specify what to do if the product leaks or spills from its container.

- On page 17 of the proposed label, add instructions to the "Pesticide Storage" section that specify what to do if the product leaks or spills from its container.

- On page 18 of the proposed label, change "connect to dispense" to read "connect to dispenser."

- Change E. coli 0157:H7 to E. coli O157:H7